Transduction of Bacteriophage Lambda by Bacteriophage T1

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When bacteriophage T1 was grown on bacteriophage λ-lysogenic cells, phenotypically mixed particles were formed which had the serum sensitivity, host range, and density of T1 but which gave rise to λ phage. T1 packaged λ genomes more efficiently both when the length of the prophage was less than that of wild-type λ and when the host cell was polylysogenic. Expression of the *red* genes of λ or the *recE* system of *Escherichia coli* during T1 growth enhanced pickup of λ by T1, whereas packaging was reduced in *recB* cells. If donors were singly lysogenic, the expression of transduced λ genomes as a PFU required λ-specified excisive recombination, whereas λ genomes transduced from polylysogens required only λ- or *E. coli*-specified general recombination to give a productive infection.

Mature phage particles of T1 and λ are morphologically similar and contain approximately the same amount of DNA (10). The life cycles of the virulent coliphage T1 and the temperate coliphage λ are completely different, and heteroduplex analysis of T1 and λ DNAs reveals no significant homology (L. A. MacHattie, personal communication). When cells are coinfected with T1 and λ, there is no detectable phenotypic mixing (11). Phage T1 is a generalized transducer and is able to transduce entire prophages, such as coliphage λ or Mu (3, 8).

With the transduction of bacterial markers, the efficiency of transduction (EOT; ratio of transductants to transducing phage) depends on the production of transducing particles, recombination between donor and recipient DNAs, and survival of the transductants. Successful transduction of prophage λ by T1 is assayed by λ plaque formation. The appearance of a λ plaque through the process of transduction by T1 depends on transducing-particle formation in the donor followed by growth and replication of the transduced prophage in the recipient. Therefore, the transduction by T1 of a given λ prophage to a given recipient cell serves as a means to identify and characterize factors which effect the production of transducing particles by T1.

In a previous report (8), evidence was presented that efficient packaging of prophage λ by T1 depends on an essential site for efficient packaging (the *esp* site) located between the galactose operon and the bacterial attachment site for λ. Data will be presented here which indicate that efficient production of a T1 particle containing a λ genome also depends on the

length of the λ prophage, the number of prophages in the donor, and the functioning of certain λ-specified and *Escherichia coli*-specified general recombination systems. In addition, once a λ genome has been transferred to a recipient by T1, efficient production of infective centers depends on the products of the *int* and *xis* genes of λ; both genes are required for site-specific, excisive recombination. In the absence of λ-specified excisive recombination, a small but significant number of infective centers are formed that depend on general recombination specified by *E. coli*.

MATERIALS AND METHODS

Bacterial and phage strains. Table 1 lists the bacterial strains used in these experiments. In the footnote to Table 1, descriptions of the various phenotypes and genotypes are give. The method used to describe genotypes is that of Bachmann et al. (1).

Table 2 lists the bacteriophages used in these experiments. Footnote *a* to Table 2 gives the characteristics of the various phage mutations.

Media. All the media used in these experiments have been described in previous reports (7, 19).

Preparation of phage lysates. Unless otherwise noted, lysates were prepared at 25°C by the standard plate-lysate method previously described (7, 19). In certain instances, which have been noted, lysates were made at 35°C, on broth cultures of cells or via single-cycle growth. With respect to lysates made at a particular temperature on a given strain, we never found a significant difference in EOT between those made in broth and those made on plates or between single and multiple cycles. After preparing a lysate, the progeny phage were mixed with W3350 *tonA*; λ phage, but not T1, will adsorb to W3350 *tonA* and after adsorption may be removed by low-speed centrifugation (e.g.,

TABLE 1. *E. coli K strains*[a]

Designation	Pertinent genotype/phenotype	Source	Reference
KB-3	*supE*	W. Michalke	20
W3350	Su⁻	E. Six	4
W3350/1	Su⁻ *tonA*		7
N100	Su⁻ *recA*	M. Gottesman	14
JC2918	Rec⁺ *supE44*	A. J. Clark	
JC2926	*recA13*	A. J. Clark	
JC5743	*recB21*	A. J. Clark	2
JC5495	*recA13 recB21*	A. J. Clark	
JC8679	*recB21 recC22 sbcA23*	A. J. Clark	
JC8682	*recA76 recB21 recC22 sbcA23*	A. J. Clark	
JC7623	*recB21 recC22 sbcB15*	A. J. Clark	18
JC7903	*recA140 recB21 recC22 sbcB15*	A. J. Clark	
JC8111	*recB21 recC22 recF143 sbc-15*	A. J. Clark	

[a] The *supE* strains are referred to in the text as Su⁺; the *supE* mutation causes the production of a tRNA species which reads the amber (*am*) codon (i.e., UAG) and inserts glutamine into the growing polypeptide chain. Su⁻ cells, such as W3350, cannot suppress *am* mutations. For a more complete description of the various *rec* systems and pathways of *E. coli*, the reader is referred to recent review articles (5, 9). Here, the *rec* systems are described in a very brief fashion. In Rec⁺ cells, about 97% of general recombination is catalyzed by the product of the unmutated *recB* and *recC* genes (i.e., by the exonuclease V). In cells which are mutants for *recB* or *recC*, the resulting deficiency in recombination can be overcome by secondary mutations in either the *sbcA* or the *sbcB* gene. The *sbcA* mutation is believed to activate or stimulate the *recE* system, whose chief product is the exonuclease VIII. The *sbcB* mutation causes a decrease in the production of the exonuclease I, and the decrease appears to permit increased activity by the unmutated product of the *recF* gene, thus restoring a high level of general recombination. All three *rec* systems mentioned here (B and C, E, F) are totally inactive with respect to recombination of bacterial DNA in cells which contain the *recA* mutation. All strains whose designations begin with JC have the *supE44* mutation.

5,000 rpm for 10 min). In some instances, anti-λ sera were used to neutralize λ.

Transduction of PFU by T1 lysates. T1 lysates can be mixed with indicator cells before plating, or the cells and phage can be mixed at the time of plating; the results are indistinguishable. About 5×10^7 indicator cells were added to 2.5 ml of soft agar together with no more than 1×10^7 PFU of T1 and poured over an agar plate; thus, the multiplicity of infection was never greater than 0.2. The plates were incubated for 18 to 24 h at 35°C. The EOT was calculated as the concentration of λ PFU assayed on T1-sensitive Su⁻ cells minus the concentration assayed on W3350 *tonA* cells, with the difference being divided by the concentration of T1 assayed on KB-3 Su⁺ cells.

Characterization of single lysogens and polylysogens. The phage λcI90c17 will form plaques on single lysogens at much lower concentrations than on polylysogens (22). The simplest way to test for single lysogeny and polylysogeny is to make a dilution series of λcI90c17 and spot portions of each dilution on lawns of a known single lysogen, a known polylysogen, and an unknown strain. Comparison of the patterns of clearing allows one to distinguish whether unknown strains are singly lysogenic or polylysogenic.

RESULTS

Transduction of λ by T1. The methods for recognizing and characterizing phenotypic mixing between λ and T1 have been described in a previous report (11). Therefore, no data are presented in this section. Instead, we merely describe the phenotypically mixed particles.

After growth of T1 on λ lysogens, λ phages were produced by cells infected with particles which are sensitive to anti-T1 sera, resistant to anti-λ sera, not able to adsorb to T1-resistant cells, and able to adsorb to λ-resistant cells. We were unable to detect any T1 phages which arose from particles which were sensitive to anti-λ sera, resistant to anti-T1 sera, able to adsorb to T1-resistant cells, and unable to adsorb to λ-resistant cells.

In a previous report we used cesium chloride density gradient centrifugation to demonstrate that the density of T1 is 1.505 g/cm³ (19). The density of T1 transducing particles is slightly, but consistently, greater than the density of T1 PFU (19). We found in this work, using previously established techniques (19), that the density of λ is 1.500 g/cm³ and that that of λ containing the *b515* and *b519* deletions is 1.489 g/cm³. The density of a T1 particle carrying either a λ or a λ*b519b515* genome is slightly greater than that of T1 PFU. Furthermore, the density of λ progeny which arose from a T1 particle carrying a λ or λ*b519b515* genome was 1.500 or 1.489 g/cm³, respectively (data not presented).

In summary, particles carrying λ genomes were detected which had the serum sensitivity, host range, and approximate density of T1. Therefore, T1 is able to package and transduce λ. The progeny λ which resulted from T1 transduction of λ had the serum sensitivity of λ, the

host range of λ, and the characteristic density of the particular λ which was transduced.

Effect of prophage length and number on transduction of λ PFU by T1. Lysates of T1 were prepared on cells lysogenic for λ. It was observed (Table 3) that when donors were lysogenic for a single, normal-length λ, the EOT was about 2×10^{-7}. With donors lysogenic for λ*b*519*b*515 (this phage contains two deletions which make it about 10% shorter than wild-type λ; see Table 2), the EOT was about 1×10^{-5}.

The EOT of λ PFU by T1 grown on polysogens of wild-type λ was more than 100-fold greater than that for lysates prepared on single lysogens. There was no significant difference between the EOTs by T1 grown on polylysogens of wild-type λ and of λ*b*519*b*515.

The data indicate that the probability of T1 packaging a segment of bacterial DNA containing a unit length of λ is greatly increased if the λ is shorter than wild type or if the donor contains multiple copies of the prophage.

Effect of temperature during lysate formation. The EOTs of λ PFU by T1 grown on cells singly lysogenic for λ*c*I⁺, λ*c*I857, and λ*red*3*c*I857, respectively, were compared for lysates made at 25, 35, and 42°C. All the prophages contained the *b*519*b*515 deletions. The *c*I857 mutation causes the production of an immunity repressor which is inactivated at 42°C (24). In our hands, prophages with the *c*I857 mutation were stable during growth at 35°C; this was established by the observation that cells grown in broth at 25°C formed equal numbers of colonies when subsequently plated at either 25 or 35°C, whereas, in contrast, colony formation dropped more than five orders of magnitude when cells were plated at 42°C (data not presented).

The results (Table 4) show that the transduction of λ PFU by T1 grown on donors lysogenic for λ*c*I⁺ was increased slightly (3- to 5-fold) when lysates were prepared at 35 or 42 rather than at

TABLE 2. *Bacteriophage strains*[a]

Designation and mutations	Source	Reference
T1*am*5*am*11[b]		7
λ wild type	A. Campbell	4
λ*b*519*b*515	M. Gottesman	21
λ*c*I857*b*515	M. Gottesman	
λ*int*6*c*I857*b*519*b*515		
λ*xis am*6*c*I857*b*519*b*515		
λ*red*3*c*I857*b*519*b*515		
λ*int*6*red*3*c*I857*b*519*b*515		
λ*c*I90*c*17	L Enquist	22
21	M. Gottesman	16
434	S. Adhya	16
λ*imm*⁴³⁴	S. Adhya	16
λ*imm*²¹	M. Gottesman	16

[a] The amber (*am*) mutation results in the production of the UAG, nonsense codon; genes with such mutations can function only in Su⁺ (suppressor) strains. The deletions *b*519 and *b*515 do not appear to affect the growth of λ or its ability to lysogenize (6, 21). *c*I857 results in the production of an immunity repressor which is inactivated at 42°C (24). *int*6 (14) inactivates the product of the *int* gene; the product is required for both integrative and excisive recombination. *xis am*6 inactivates excisive recombination in Su⁻ but not Su⁺ cells (15). *red*3 inactivates the *red* system of general recombination (23). The *c*I90 mutation causes the production of an inactive or partially active *c*I (i.e., immunity) repressor. The *c*17 mutation creates a rightward promotor which bypasses promotion at the $o_R p_R$ site; *c*I repressor interferes with promotion at *c*17, and the interference increases with the concentration of repressor. Description of the phages 21, 434, λ*imm*⁴³⁴, and λ*imm*²¹ and other useful information about λ can be found in *The Bacteriophage Lambda* (16).
[b] Referred to as T1.

TABLE 3. *Transduction of λ PFU by T1 grown on λ lysogens*[a]

Prophage	EOT × 10⁶
λ wild type	0.2
λ*b*519*b*515	10
λ wild type (polylysogen)	50
λ*b*519*b*515 (polylysogen)	40

[a] T1 was grown on each of the singly lysogenic donors listed in the table. Each lysate was assayed for total λ PFU by the standard agar overlay method with W3350 as the indicator cells; in all platings with the W3350, the multiplicity of infection was 0.2 or less. The concentration of free λ was assayed with W3350 *tonA* as the indicator cells. The EOT was calculated by dividing the difference between the concentration of λ PFU on W3350 and the concentration on W3350 *tonA* by the concentration of T1 (obtained by utilizing KB-3 as the indicator of T1 PFU). All the lysates were prepared at 25°C.

TABLE 4. *Effect of temperature during T1 lysate preparation on transduction of λ PFU*[a]

Prophage	EOT × 10⁶ of lysate produced at:		
	25°C	35°C	42°C
λ	10	40	50
λ*c*I857	50	400	1,000
λ*red*3*c*I857	30	80	70

[a] All the prophages listed in the table contained the *b*515 and *b*519 deletions. Lysates of T1 were prepared on singly lysogenic donors. Each lysate represents a single cycle of T1 growth on broth cultures of the KB-3 lysogens. The assay method and the method of calculating the EOT are given in the footnote to Table 3.

25°C. When donors were lysogenic for λcI857, T1 lysates prepared at 35 and 42°C were about 10- and 20-fold more efficient, respectively, in transducing λ PFU than were 25°C lysates. When the donor's prophage contained the red3 mutation together with the cI857 mutation, the EOT by lysates prepared at 35 or 42°C was slightly (2- to 3-fold) greater than that by 25°C lysates. In other words, the manner in which T1 lysates prepared on donors lysogenic for λred3cI857 were affected by temperature more closely resembled the effects on lysates prepared on cells lysogenic for λcI⁺ than the effects on lysates prepared on cells lysogenic for λcI857.

In summary, during the production of T1 lysates on λ lysogens, an elevation in temperature from 25 to 35 or 42°C led to an increase in the packaging of prophage λ relative to the packaging of T1 progeny. The increased packaging of DNA which had the potential to cause formation of a λ infective center was greater when the prophage in the donor was λcI857; this further enhancement did not occur when the prophage was λred3cI857. Therefore, we believe that the λ prophage was partially derepressed at 35°C, thus permitting a limited amount of transcription of the red genes; the products of the red genes led to a relative increase in the packaging of prophage λ into T1 transducing particles. The fact that there was only a relatively small increase in the EOT of 42°C lysates prepared on cells lysogenic for λcI857 compared with 35°C lysates suggested that complete derepression (i.e., induction) of prophage λ was not necessary to achieve the relative increase of transducing-particle formation.

Formation of transducing particles in strains with rec mutations. Lysates of T1 were prepared at 25 or 35°C on a series of nearly isogenic E. coli strains which carried one or more mutations affecting cell-specified, general recombination. All of the strains were lysogenic for λcI857b519b515 (referred to simply as λ).

It was observed (Table 5) that the EOT of λ PFU by T1 grown on JC2918(λ), a strain which is wild type (i.e., Rec⁺) for all the rec and sbc genes studied here, was indistinguishable from that of T1 grown on KB-3(λ) (see Table 3). The EOTs of T1 grown on each of the various λ-lysogenic, rec mutants were compared to those of Rec⁺ JC2918(λ) (Table 5). With two exceptions, the results showed that the EOT values of T1 lysates prepared at 25°C on the various mutant strains were essentially identical or marginally reduced compared with those obtained with the JC2918(λ) Rec⁺ strain. It should be noted that, with respect to general recombination assayed by a variety of methods, strains JC8679 (recB recC sbcA) and JC7623(recB recC sbcB)

TABLE 5. Formation of λ-transducing T1 particles in E. coli rec mutants[a]

Donor strain	Genotype	EOT × 10⁶ of lysate prepared at:	
		25°C	35°C
JC2918	rec⁺	50	500
JC2926	recA	10	200
JC5743	recB	4	100
JC5495	recA recB	20	200
JC8679	recB recC sbcA	300	300
JC8682	recA recB recC sbcA	30	500
JC7623	redB recC sbcB	20	200
JC7903	recA recB recC sbcB	30	200
JC8111	recB recC recF sbcB	10	300

[a] Each of the indicated donor strains, kindly supplied by A. J. Clark, was lysogenized with λcI857b519b515 and used as a host for T1. The resulting T1 lysates, prepared at 25 and 35°C, respectively, were tested for their abilities to transduce a λ PFU to the Su⁻ Rec⁺ recipient strain W3350. The assay method and the method of calculating the EOT are given in the footnote to Table 3.

are phenotypically Rec⁺(5). Exceptions were: T1 grown at 25°C on JC5743(λ), a recB strain, consistently transduced λ PFU much less efficiently, whereas T1 grown on JC8679(λ), a recB recC sbcA strain (i.e., a strain with an activated recE system), transduced more efficiently than did T1 grown on JC2918(λ) Rec⁺.

All lysates made at 35°C transduced with high efficiency. Presumably, at 35°C the stimulation of transducing-particle formation caused by the red function of λ (described above) occurred in the absence of rec-mediated general recombination.

It was observed that T1 was able to package λ as efficiently in strains which are, essentially, totally deficient in rec-mediated recombination of bacterial DNA (i.e., in recA, recA recB, recA recB recC sbcA, recA recB recC sbcB, and recB recC recF sbcB strains) as in rec⁺ strains. However, the absence of a functional recB gene alone, in some way, impaired the ability of T1 to package λ genomes relative to the packaging of T1 genomes. The effect of the recB mutation in reducing the EOT of λ PFU could be reversed by secondary mutations, such as recA, sbcA, or sbcB. The effect of secondary mutations in restoring the EOT of T1 lysates prepared on recB strains probably did not depend solely on the activation of an alternate recombination system specified by E. coli, since recA mutations have the opposite effect and recB recC recF sbcB strains are more deficient in general recombination than are recB strains. However, in the case of the JC8679(λ) recB recC sbcA strain, the activation of the recE system was clearly re-

sponsible for the greater-than-normal EOT, since T1 grown on the JC8682(λ) *recA recB recC sbcA* strain showed only an average ability to transduce λ PFU.

Effect of site-specific and general recombination on formation of λ PFU in recipients. If λ is to be expressed as a PFU in the recipients, it must replicate, and the first step in this process is the formation of a circle. When packaged by T1, λ DNA presumably lacks the cohesive ends that normally provide for this step. Since recombination potentially provides an alternate route to circular DNA, we have explored the role of *int⁻*, *xis⁻*, *red⁻*, and *rec*-promoted recombination on the transduction of λ PFU. Lysates prepared on donors singly or multiply lysogenic for λ carrying *int*, *xis*, or *red* mutation(s) were tested on *rec⁺* and *recA* recipients. Results are in Table 6.

Lysates made on polylysogens were considered first. Potentially, any complete λ genomes packaged from the bacterial chromosome by T1 could have contained a considerable length of terminal redundancy of λ DNA; this arises from the fact that the prophages contained the *b*515 and *b*519 deletions. The length of the redundancy would depend on where the packaging was initiated and terminated. Initiation and termination within a λ genome would lead to a particle which contained the *attP* site and a maximum amount of terminal redundancy (see Fig. 1a). Initiation or termination within bacterial DNA during the packaging of a complete λ genome would result in a particle containing ether *attL* and *attP* or *attP* and *attR* together with a relatively smaller amount of terminal redundancy than that in the particles described
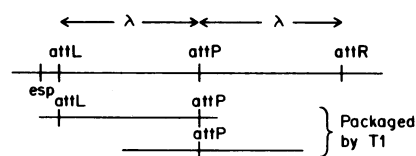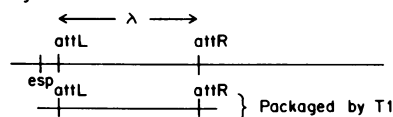

FIG. 1. *Region of the E. coli chromosome where λ integrates. esp is a site at which T1 has been shown to efficiently initiate packaging of DNA in a unidirectional (toward attλ) fashion (8). Beneath the map segments are possible configurations of the form of prophage λ packaged into T1 transducing particles.*

above. Utilizing repeated sequences of λ, the *red* system of λ or the *rec* systems of *E. coli* could promote circle formation for either particle, whereas site-specific recombination could probably promote circulation only for DNA containing two attachment sites.

The importance of recombination for the transduction of λ PFU is shown by the large reduction in the EOT of λ*intred* phages in *recA* recipients. The presence of any of the three recombination pathways, *int*, *red*, or *rec*, restored the EOT to very near that obtained with λ*b*519*b*515 polylysogenic donors and *rec⁺* recipients. Although the precision of the data is probably not sufficient to allow firm conclusions about the relative importance of each pathway, it would appear that the single deficiency of *int* is least important, since the λ*int* lysates transduced to either a *rec⁺* or a *recA* recipient with high efficiency. Nevertheless, *int* alone was capable of promoting expression of λ PFU to a rather high level, as shown by the transduction of λ*red* to *recA* recipients.

Transduction from singly lysogenic donors gives an entirely different picture. The packaging of prophage DNA directly from the chromosome of a singly lysogenic strain would not give any terminal redundancy, but when a complete prophage was packaged, it would contain both *attL* and *attR* (Fig. 1b). Thus, a complete prophage could form a circle through the activities of the products of the *int* and *xis* genes (i.e., through site-specific, excisive recombination).

It was found (Table 6) that the presence of either the *int* of the *xis* mutation did reduce the EOT from singly lysogenic donors to *rec⁺* recipients at least 10-fold, compared with *int⁺* and *xis⁺* (see Table 3). The use of phages with the *red* mutation caused no additional reduction in

TABLE 6. *Effect of int and red mutations on transduction of λ PFU from singly lysogenic and polylysogenic donors*[a]

Donor prophage	Lysate preparation temp (°C)	EOT × 10⁶ with recipient phenotype:	
		Su⁻ Rec⁺	Su⁻ Rec⁻
Polylysogenic			
λ*int6*cI857	25	40	20
λ*red3*cI857	25	20	8
λ*int6red3*cI857	25	6	0.09
Singly lysogenic			
λ*int6*cI857	25	0.3	0.01
λ*int6*cI857	35	3	0.08
λ*xisam6*cI857	25	1	0.4
λ*int6red3*cI857	25	1	0.04

[a] All the prophages listed in this table contained the *b*515 and *b*519 deletions. T1 was prepared on the singly lysogenic and polylysogenic donors listed in the table at the temperatures indicated. The assay method and the method of calculating the EOT are given in the footnote to Table 3.

EOT, but the use of *recA* recipients led to a reduction of the EOT to values which were at least 100-fold lower than those obtained with phages having functional *int* and *xis* genes (see Table 3). Lysates prepared at 35°C on donors singly lysogenic for λ*int* showed the typical 10-fold increase that is characteristic of lysates grown on donors whose prophage is *red*⁺ (see Tables 3 and 6). It should be noted that the *xisam6* mutation is suppressed in the KB-3 Su⁺ donor cells; this supports the idea that the reduction in the EOT caused by a deficiency in site-specific recombination is due to the lack of site-specific recombination in the W3350 Su⁻ recipient cells.

We conclude that *red-* or *rec*-promoted recombination in recipient cells permitted maximum PFU expression by λ genomes transduced from polylysogenic donors. In the absence of *red-* or *rec*-promoted recombination, expression of *int* restored the EOT to levels somewhat lower than *red* or *rec* but, nevertheless, to a relatively high level. Either *int* or *xis* was required for efficient expression of transduced λ DNA as PFU when the donor was singly lysogenic. In the absence of *int* or *xis* function, a further reduction in EOT occurred when recipient cells were *recA*. The *red* function of λ appeared to have no effect in recipient cells on the EOT or λ PFU from singly lysogenic donors.

Transduction of other lambdoid phages by T1. T1 was grown on cells lysogenic for the lambdoid phages 21 and 434 and the hybrid phages λ*imm*²¹ and λ*imm*⁴³⁴, respectively. The results (Table 7) demonstrated that T1 was able to transduce all the phages tested. Since we had no method of determining whether the strains were singly or multiply lysogenic, we were not able to determine whether the observed variability in EOT was due to the numbers of prophages present in the donors.

DISCUSSION

During the growth of T1 on cells lysogenic for λ, particles were formed which gave rise to λ PFU but had the host range, density, and neutralization characteristics of T1. These T1 transducing particles were made more than 100 times more efficiently if the length of the λ prophage was less than that of the wild type or if the donor was polylysogenic for λ. When either the *red* system of λ or the *recE* system of *E. coli* was expressed during the production of T1 lysates, the lysates had an increased EOT for λ PFU. Inactivation of the product of the *recB* gene of donor cells led to decreased formation of T1 particles carrying λ PFU, except in cells which were, in addition, mutants in either *recA*, *sbcA*,

TABLE 7. *Transduction of phages related to λ by T1*[a]

Prophage in the donor KB-3 strain	EOT × 10⁶
21	0.4
434	3
λ*imm*²¹	50
λ*imm*⁴³⁴	30

[a] All the prophages listed in the table integrate at the *att*λ site except for phage 21, which integrates at the attachment site for φ80 (*att*φ80). Phages were kindly supplied by M. E. Gottesman. The assay method and the method of calculating the EOT are given in the footnote to Table 3.

or *sbcB*. For efficient formation of infective centers, the λ genomes which were packaged into T1 transducing particles required recombination in recipient cells. The type of recombination required for infective centers to be formed by λ genomes which were transferred by T1 depended on the previous host. λ genomes packaged by T1 during growth on singly lysogenic donors required site-specific, excisive recombination for efficient formation of infective centers; λ genomes packaged in polylysogenic cells appeared to be of two types, one being able to use either site-specific recombination or general recombination and the other being able to use only general recombination.

The observations presented in this report together with results reported in a previous publication (8) lead us to conclude that when T1 was grown on cells singly lysogenic for λ, the prophage form of λ was packaged in T1 transducing particles. In other words, we believe that a stretch of the bacterial chromosome containing the λ prophage was packaged by T1 (see Fig. 1b); after attachment to appropriate recipient cells, the T1 transducing particles caused the formation of λ infective centers. In a previous report (8) it was shown that a site on the bacterial chromosome (the *esp* site) located between the galactose operon and the attachment site for λ (*att*λ) enhanced the pickup of bacterial DNA in a polar (i.e., unidirectional) fashion toward the *att*λ site. The *esp* site enhanced efficient packaging of only those markers closely linked to it and stimulated pickup of both the biotin operon and prophages located at *att*λ. Thus, the data suggested that λ had to be linked to the bacterial chromosome for efficient packaging by T1 to take place. In this report, it was demonstrated that efficient production of infective centers by transduced λ genomes required λ-specified, excisive recombination in the recipient. Since the vegetative cycle of λ begins with the formation of a circular chromosome, the sim-

plest explanation of the requirement for excisive recombination is that it is needed for circularization of the transduced λ genome—in other words, the λ prophage must "excise" from the bacterial DNA to which it is covalently coupled. Thus, infective center formation by transduced λ is formally analogous to the zygotic induction of prophage λ which is a consequence of conjugation between F$^+$ (λ) and F$^-$ cells (17).

When T1 was grown on polylysogenic donors, none of the transduced λ genomes seemed to require excisive recombination. About one-third to one-half of the λ genomes packaged during growth of T1 on polylysogens were able to form infectious centers through either λ-specified site-specific recombination, λ-specified general recombination, or *E. coli*-specified general recombination; the remaining particles were able to use only phage- or cell-specified general recombination. The simplest explanation of these results was that one-third to one-half of the λ genomes transduced from polylysogens possess two phage attachment sites and repeated sequences of homologous DNA (i.e., redundancy), whereas the remaining λ genomes possess repeated sequences but only one attachment site; Fig. 1a demonstrates a plausible explanation for the packaging of λ genomes from polylysogens, but other explanations are possible.

When lysates are prepared at 35°C, an activated *recE* pathway does not seem to enhance donor activity beyond that already provided by partial expression of the *red* genes. This suggests that the *recE* and λ *red* functions act in a similar way in their enhancement of the transduction of λ by T1; otherwise, an additive effect would likely have been observed.

Several reports by others have suggested that the product of the *red* gene of λ and exonuclease VIII of *E. coli* (exonuclease VIII is part of the *recE* system) are functional isozymes (5, 13). The known nucleolytic or recombination activities of *red* and *recE* products offer no clue as to what role they played in altering the ratio of T1 transducing particles carrying λ PFU and progeny T1. On the one hand, production of transducing particles could potentially be increased or decreased through an alteration in the specificity of packaging. On the other hand, interference with the supply of bacterial or phage DNA available for packaging could also change the ratio of transducing particles and T1 PFU. Thus, the activity of *red* or *recE* might be at the level of recognition of packaging sites, interference with breakdown of the bacterial chromosome, or impairment of the replication of T1 DNA. A previous report has shown that λ *red* can substitute for the function of T1 gene 4 (11), and it

has recently been shown that *recE* function can substitute for both gene 4 and the related gene 3.5 (J. R. Christensen, L. Bourque, and D. A. Ritchie, unpublished data). T1 packages its DNA from concatemers, starting from a unique site (12). Gene 4 mutants fail to make concatemrs (D. A. Ritchie, personal communication). One final commnt concerning *recE* and λ *red*: the effect of *recE* reported here required a functional *recA* gene; thus, the effect resembled the activity of *recE* in promoting bacterial recombination, which requires *recA*, rather than its activity in promoting recombination between λ*red* phages, which does not (5).

The EOT of λ PFU by T1 is reduced when the donor cell is *recB*. The reduction in EOT may occur for one of several reasons. For example, *E. coli* products other than *recB* may convert the packaging substrate to an intermediate which can be efficiently packaged only by a functional *recB* product. Alternatively, the packaging intermediate may be prevented from being packaged by T1 products as a result of efficient competition by the nonfunctional *recB* product. Both explanations suggest that exonuclease V, which is the product of the *recB* and *recC* genes, can affect the specificity of packaging. However, as with the *red* and *recE* effects (see above), unknown effects on degradation of the bacterial chromosome or replication of T1 DNA may result from the *recB* mutation. The fact that mutants in *recA* (required for all known bacterial recombination) can reverse the effect of *recB* mutations clearly implies that T1 itself is able to produce products which are fully able to package bacterial or T1 DNA.

An interesting observation was that a small fraction of the λ genomes transferred by T1 grown on singly lysogenic donors was able to utilize the *rec* system to form infective centers. If some λ genomes were occasionally packaged from multimers of λ, T1 might package terminally redundant genomes which could circularize by means of homology-dependent, general recombination; this is what we believe occurred when T1 grew on polylysogens (see Fig. 1a). However, the λ *red* system seemed unable to replace the *rec* system in *recA* cells. Therefore, the *rec* system seems to be catalyzing infective center formation by a mechanism not open to the *red* system.

With regard to the form of λ packaged, this report indicates that transduction of λ genomes by T1 is not the result of induction of λ followed by packaging of λ genomes by T1 from concatameric λ. Furthermore, λ is not packaged in its normal configuration, with complementary, single-stranded ends. Although the data support

the idea that the initiation of packaging of λ in single lysogens is at a bacterial site, thus leading to pickup of the prophage form of λ, at least two sites of initiation for head filling must be used efficiently in polylysogens.

At present, it seems obvious that the interaction of recombination systems and nucleases specified by T1, λ, and *E. coli* can affect packaging in a very complicated manner which alters the ratio of transducing particles to PFU. This work has provided a simple and reproducible method of assaying events in the donor that affect production of transducing particles. Hopefully, future experiments will enable us to gain more understanding of the packaging process.

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